REMARKS

The above amendment is made to place the present application into close conformity with U.S. practice and to complete certain informalities that are prerequisite to entry into the National Phase before the U.S. Patent and Trademark Office. A Sequence Listing in conformity with U.S. practice is submitted herewith together with a computer-readable copy and the necessary Declaration confirming that both are identical to each other. No new matter is inserted hereby, as the Sequence Listing in question corresponds in substance to the listing that was present in the parent International Application.

Also, appropriate amendments have been made in the Specification where reference to the sequences by other than sequence listing identification, is present. Thus, the paragraphs set forth in the amendment to the Specification identify those passages where reference to a Sequence Listing appears necessary.

Lastly, the Claims have been amended to include reference to the Sequence Listing, where appropriate. Likewise, the claims have been revised to place them into greater conformity with U.S. practice by eliminating improper multiple dependencies and the redrafting of certain claims, such as, for example, the revision of Claims 30, 31 and 33 to convert them from use claims, and the presentation of new Claims 34-39 to cover appropriately worded methods, both diagnostic and therapeutic.

The Claims presented by this amendment are believed to be in proper form for U.S. practice, and entry and favorable consideration thereof are requested.

Entry of the foregoing amendments and early and favorable processing in the National Phase before the United States Patent and Trademark Office is courteously solicited.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The Specification has been amended as follows:

Please replace the paragraph at Page 2, Lines 23-26, with the following

--According to a first aspect of the present invention there is provided a recombinant protein that exhibits significant sequence homology with the tick-derived protease inhibitor protein (TdPI) sequence given in Figure 1 (SEQ.ID. NO:2), an active fragment of said protein or a functional equivalent of said protein.--

Please replace the paragraph at Page 3 Lines 7-14, with the following

--Included in this aspect of the invention there is provided a protein comprising the sequence identified herein as tick-derived protease inhibitor protein (TdPI), an active fragment thereof or a functional equivalent thereof. This sequence is given in accompanying Figure 1 (SEQ.ID.NO:2). This protein was identified as being encoded by a cDNA from a tick salivary gland library. The protein has a molecular weight of approximately 13.5 kDa and appears to belong to the family of Kunitz-type protease inhibitors. The sequence similarity with other members of this family such as aprotinin and inter-alpha-trypsin inhibitor is low, but the putative reactive centre and the position of the cysteines is to some extent conserved.--

Please replace the paragraphs at Page 8, Lines 20-22 and 23-29, respectively, with the following

--A cDNA encoding TdPI is disclosed herein by way of example and its sequence and the amino acid sequence it encodes are shown in Figure 1 (nucleotides (SEQ ID NO:1)and amino acids (SEQ ID NO:2)are given in their standard one letter abbreviations).

A preferred nucleic acid molecule according to the invention comprises a nucleotide sequence identical to or complementary to the sequence shown in Figure 1(SEQ ID NO:1), or a sequence that is degenerate or substantially homologous therewith, or which hybridises with this sequence under non-stringent conditions, for instance 6 x SSC/50% formamide at room temperature, and washed under conditions of low stringency, for instance (2 x SSC room temperature or 2 x SSC, 42°C or, more preferably, binding under conditions of higher stringency, e.g. 2 x SSC, 65°C. (SSC = 0.15M NaCl, 0.015M sodium citrate, pH 7.2).--

Please replace the paragraphs at Page 10, lines 19-20 and 23-26, respectively, with the following:

--Figure 1 shows the cDNA sequence (SEQ ID NO:1) and inferred amino-acid sequence (SEQ ID NO:2)of TdPI-encoding clone 76-3.

Figure 3 shows an alignment of TdPI (SEQ ID NO:2) with Kunitz domains of the bovine colostrum trypsin inhibitor (SEQ ID NO:3) (BovCol; Cechova, 1976), (bovine) aprotinin (SEQ ID NO:5) (Creighton & Charles, 1987), and the rat tissue factor pathway inhibitor (TFPI-2 (SEQ ID NO:4); only the second, factor Xa-inhibiting domain is shown; Enjyoji *et al.*, 1992).--

Please replace the paragraph from Page 12, Line 16 to Page 13, line2, with the following:

--Recombinant TdPI (rTdP1) was expressed as a histidine-tagged protein in *Spodoptera frujiperda* ovarian cells (SJ21; Invitrogen). The coding region of the TdPI cDNA was amplified by the polymerase chain reaction (PCR), using the forward primer

5'-GCAGGAGCTCGGCACGAG (SEQ ID NO:9)

and the reverse primer

5'-TATGGATCCCAGGTCCAGGCTCTGTTCCG (SEQ ID NO:10),

thereby adding a *Sac* I site upstream of the start codon, and replacing the stop codon with a *Bam* HI site. The PCR consisted of 20 cycles with a 30-second melting step (95"Q a 30-second primer-annealing step (50°C) and a 30-second extension step (72°C). The PCR product was ligated between the *Sac* I and *Bam* HI sites of the pAC129.1 transfer vector (Livingstone & Jones, 1989), which was modified so that a carboxyterminal Gly-Ile-$(His)_6$ tag was added to the expressed protein. Co-transfection of SJ21 cells with the transfer vector and baculovirus (BacPak6) and amplification of recombinant virus was as described by Kitts & Possee, 1993. rTdPI was expressed in TC100 medium (Gibco BRL) containing 10% foetal bovine serum (Sigma).--

Please replace the paragraph from Page 14, Lines 4-16 with the following:

--Figure 3 shows an alignment of TdPI (SEQ ID NO:2) with Kunitz domains of the bovine colostrum trypsin inhibitor (SEQ ID NO:3) (BovCol; Cechova, 1976), (bovine) aprotinin (SEQ ID NO:5) (Creighton & Charles, 1987), and the rat tissue factor pathway inhibitor (SEQ ID NO:4) (TFPI-2; only the second, factor Xa-inhibiting domain is shown; Enjyoji *et al.*, 1992). The Kunitz domains of the tick anticoagulant peptide TAP (SEQ ID NO:6) (Waxman *et al.*, 1990) and the two domains in ornithodorin (ornithl (SEQ ID NO:7) and ornith2 (SEQ ID NO:8); Van de Locht *et al.*, 1996) are also included. The alignment of TdPI with the vertebrate Kunitz domains was created using GCG's "pileup" and "prettyplot" commands, choosing relatively low gap and length weights (1 and 0.03, respectively). The alignment was then modified, mainly by introducing extra gaps, so that the TAP and ornithodorin domains could be included. The modification was largely based on the alignment of the latter domains with aprotinin, as reported by Van de Locht *et al.*, 1996. The arrow indicates the PI residue of the aprotinin binding loop. The asterisks denote the cysteines involved in disulphide-bridge formation in traditional Kunitz domains.--

IN THE CLAIMS:

Claims 1, 3, 4, 7, 10-14, 16-24, 27, and 29-33 have been amended as follows:

1. (Amended) A recombinant protein that exhibits significant sequence homology with the tick-derived protease inhibitor protein (TdPI) sequence [given in Figure 1] set forth in SEQ ID NO:2, an active fragment of said protein or a functional equivalent of said protein.

3. (Amended) A recombinant protein, protein fragment or functional equivalent according to [either of] claim[s] l[-2] that contains one or more epitopes that can be used in the development of vaccines that target proteins that exhibit significant sequence homology with TdPI.

4. (Amended) A recombinant protein or protein fragment according to claim I, wherein said sequence homology is defined as 50% or more of the amino acids in the sequence being completely conserved as identical residues if the protein is aligned with the sequence of [Figure 1] SEQ ID NO:2, the alignments being obtained using GCG's bestfit command (gap creation penalty = 2.5; gap extension penalty = 0.5).

7. (Amended) A recombinant protein or protein fragment according to [any one of claims 1-6] claim 1 comprising the TdPI sequence.

10. (Amended) A recombinant protein, protein fragment or functional equivalent according to claim 8 [or claim 9] that contains one or more epitopes that can be used in the development of vaccines that target proteins that exhibit significant sequence homology with TdPI.

11. (Amended) A recombinant protein or protein fragment according to either [any one] of claims l[-10] or 8 that inhibits tryptase with a Ki of less than 1×10^{-6} M, preferably less than 1×10^{-7} M, more preferably less than 2×10^{-8} M, most preferably less than 1×10^{-9} M.

12. (Amended) A recombinant protein, protein fragment or functional equivalent according to either of [any one of the] claims 1[-1 1] or 8 that inhibits catalytic tryptase activity.

13. (Amended) A recombinant protein, protein fragment or functional equivalent according to either of [any one of] claims 1[-12] or 8 which inhibits mast cell tryptase, preferably human mast cell tryptase.

14. (Amended) A recombinant protein, protein fragment or functional equivalent according to [any one of the preceding claims] claim 1, that is derived from a tick.

16. (Amended) A recombinant protein, protein fragment or functional equivalent according to [any one of the preceding claims] either of claims 1 or 8 that has been genetically or chemically fused to one or more peptides or polypeptides.

17. (Amended) A recombinant protein, protein fragment or functional equivalent according to [any one of the preceding claims] either of claims 1 or 8 that is bound to a support, such as a resin.

18. (Amended) A pharmaceutical composition comprising a recombinant protein, protein fragment or functional equivalent according to [any one] either of claims 1[-17] or 8, in conjunction with a pharmaceutically-acceptable carrier.

19. (Amended) A vaccine composition comprising a recombinant protein, protein fragment or functional equivalent according to [any one] either of claims 1[-15] or 8, optionally in conjunction with an adjuvant.

20. (Amended) A process for the formulation of a pharmaceutical composition according to claim 19 comprising bringing [a] said recombinant protein, protein fragment or functional

equivalent [according to any one of claims 1-15] into association with a pharmaceutically-acceptable carrier.

21. (Amended) A recombinant protein, protein fragment or functional equivalent according to [any one] either of claims 1 [to 15] or 8 for use as a pharmaceutical.

22. (Amended) A method for the prevention or treatment of a disease in a subject, comprising administering to said subject an effective dose of a composition according to claim 18 [or claim 19].

23. (Amended) A nucleic acid molecule encoding a recombinant protein, protein fragment or functional equivalent according to [any one of claims 1-16] claim 1.

24. (Amended) A nucleic acid molecule[:] having the sequence [given in Figure 1] set forth in SEQ ID NO:1; which hybridises with said nucleotide sequence under stringent hybridisation conditions; or which encodes on expression a recombinant protein, protein fragment or functional equivalent as defined in [any one of claims 1-16] claim 1.

27. (Amended) A host cell transformed or transfected with the vector of claim 25 [or claim 26].

29. (Amended) A method of preparing a recombinant protein, protein fragment or functional equivalent [according to any one of claims 1 to 16], comprising expressing a vector according to claim 25 or claim 26 in a host cell and culturing said host cell under conditions where said recombinant protein, protein fragment or functional equivalent is expressed, and recovering said recombinant protein, protein fragment or functional equivalent thus produced.

30. (Amended) [Use of a recombinant protein, protein fragment or functional equivalent according to any one of claims 1-17 for:]A method for the detection or quantification of

tryptase[; for the depletion or removal of tryptase from a food product or from a cell culture; as an anti-tryptase agent; or as an anti-inflammatory drug] in a sample to be tested, comprising contacting said sample with a kit comprising at least one recombinant protein, protein fragment or functional equivalent according to claim 1, and other reagents for detection.

31.(Amended) [Use of a recombinant protein, protein fragment or functional equivalent according to any one of claims 1 to 16 in the manufacture of a medicament] A method for the treatment of inflammation in humans or animals comprising administering a therapeutically effective amount of a recombinant protein, protein fragment or functional equivalent according to claim 1.

32. (Amended) A method of vaccinating a mammal against a disease, or of treating a mammal suffering from a disease, comprising administering a recombinant protein, protein fragment or functional equivalent according to [any one of claims 1 to 16] claim 1 to a said mammal.

33. (Amended) [Use of] A tryptase inhibitor comprising a protein or protein fragment selected from the group consisting of bovine colostrum trypsin inhibitor, the rat tissue factor pathway inhibitor (TFPI-2), the Kunitz domain of the tick anticoagulant peptide TAP and the two domains in omithodorin [as a tryptase inhibitor].

The following new Claims 34, 35, 36, 37, 38 and 39 have been added.

--Claim 34. A method for the prevention or treatment of a disease in a subject, comprising administering to said subject an effective dose of a composition according to claim 19. --

--Claim 35. A host cell transformed or transfected with the vector of claim 26.--

--36. A method for the depletion or removal of tryptase from a food product or from a cell culture comprising contacting the food product or cell culture with a quantity of a recombinant protein, protein fragment or functional equivalent according to claim 1. --

--37. The method of claim 36 wherein said recombinant protein, protein fragment or functional equivalent is bound to a support.--

--38. An anti-tryuptase agent comprising a recombinant protein, protein fragment or functional equivalent according to claim 1.--

--39. An anti-inflammatory agent comprising a recombinant protein, protein fragment or functional equivalent according to claim 1. --